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Reproductive rate, not dominance status, affects fecal glucocorticoid levels in breeding female meerkats

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ABSTRACT

Glucocorticoid hormones (GCs) have been studied intensively to understand the associations between physiological stress and reproductive skew in animal societies. However, we have little appreciation of the range of either natural levels within and among individuals, or the associations among dominance status, reproductive rate and GCs levels during breeding. To address these shortcomings, we examined variation in fecal glucocorticoid metabolites (fGC) during breeding periods in free-ranging female meerkats (*Suricata suricatta*) over 11 years. The vast majority of variation in fGC levels was found within breeding events by the same female (~87%), with the remaining variation arising among breeding events and among females. Concentrations of fGC generally tripled as pregnancy progressed. However, females with a high reproductive rate, defined as those conceiving within a month following parturition (mean = 9 days postpartum), showed significant reductions in fGC in the final 2 weeks before parturition. Despite these reductions, females with a high reproductive rate had higher fGC levels at conception of the following litter than those breeding at a low rate. After controlling for the higher reproductive rate of dominants, we found no association between levels of fGC and either age or dominance status. Our results suggest that one should be cautious about interpreting associations between dominance status, reproductive skew and GCs levels, without knowledge of the natural variation in GCs levels within and among females.

Introduction

Individual fitness is typically maximized through independent reproduction. In species that breed cooperatively, however, individuals commonly forego personal reproduction and instead contribute to rearing the offspring of others (Choe and Crespi, 1997; Hager and Jones, 2009; Solomon and French, 1997; Stacey and Koenig, 1990; Wilson, 1971). Given that in such systems dominant breeders gain through the helping actions of non-breeding subordinates, the former should be under strong selection to reduce the reproductive rate of the latter. In some social insects, for example, queens use pheromones to signal their presence and workers are often born sterile, while, those that are not, risk personal injury and egg destruction (Beekman and Ratnieks, 2003). In vertebrates, where all offspring are born with reproductive capabilities, subordinate reproduction is reduced, in part, by evicting them from the group and/or killing their offspring (Cant, 2011; Koenig and Dickinson, 2004). However, whether or not dominants also attempt to physiologically suppress the reproductive system of subordinates or whether subordinates depress their own reproductive system when their chances of success are low, is contentious (Creel, 2001; Koenig and Dickinson, 2004; Young et al., 2006).

In vertebrates, glucocorticoids (GCs) play a key adaptive role in metabolic, immune, behavioral and reproductive functions (de Kloet et al., 1999; Dhabhar, 2002; Magiakou et al., 1997; Whittle et al., 2001; Wingfield and Kitaysky, 2002). However, at chronic elevations, they can reduce survival and reproductive functions (Sapolsky et al., 2000; Selye, 1956). For example, sustained elevations in GCs can render females infertile by suppressing gonadal activity (Bennett, 1994; Nakamura et al., 2008) and have been suggested to lead to increased risk of abortion (Saltzman et al., 2006; Young et al., 2006). The link between stress and female reproductive failure has led to the hypothesis that chronic elevations in GCs can provide a proximate mechanism for understanding skews in the proportion of breeding versus non-breeding group members in some vertebrate societies (Creel, 2005). Hypotheses of reproductive skew based on dominance-mediated physiological suppression versus personally-mediated reproductive restraint

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have opposing predictions regarding GCs levels in dominant versus subordinate females. Under the former, subordinates would be expected to have significantly higher GCs levels than dominants, while this should not be the case under the latter (Creel, 2001, 2005; Young et al., 2006, 2008).

Creel (2001) rejected a role of physiological suppression in cooperative vertebrates based on findings that subordinates seldom have higher GCs levels than dominants across species. For instance, experimental evidence on the role of social stress in common marmosets, *Callithrix jacchus* (Abbott et al., 1997), and naked mole-rats, *Heterocephalus glaber* (Faulkes and Abbott, 1997), failed to reveal a link between GCs and infertility. Young et al. (2006), however, cautioned against premature rejection of the physiological suppression hypothesis, if dominants only induce high levels of stress in subordinates during particular times. In support, subordinate meerkats (*Suricata suricatta*) were shown to have significantly elevated GCs levels when evicted from the group, commonly aborted litters as a consequence of eviction and failed to conceive when evicted despite the presence of unrelated males (Young et al., 2006). Importantly, these results were found despite the finding that there was no difference in GCs levels between dominant and subordinate female meerkats during periods of non-breeding (Young et al., 2008).

One of the problems of interpreting the role of adrenal functions in mediating reproductive skew is that we know little about natural variation in GCs within- and among-individuals and the individual-specific levels required to induce reproductive suppression (Koolhaas et al., 1999; Romero and Reed, 2008; Sapolsky, 1994, 1999; Williams, 2008). This is compounded by the fact that most previous studies have been conducted during non-breeding periods to avoid the confounding effects of increased adrenal activity during breeding (Brunton et al., 2008; Dantzer et al., 2010; Krasnow and Steiner, 2006; Mastorakos and Ilias, 2003; Trainer, 2002). Longitudinal studies making use of non-invasive assessments of GCs excretion across breeding and non-breeding periods are now required to clarify patterns of natural variation (Bonier et al., 2009; Dingemanse et al., 2010). In particular, such studies need to include breeding periods for a full understanding of links among reproductive success, dominance status and GCs levels. For example, it is difficult to judge levels of GCs that might prevent reproduction or induce abortion, if we do not know natural levels associated with conception and gestation. In addition, GCs levels might not only mediate qualitative differences in breeding propensity, but also quantitative differences in breeding success between dominant and subordinate individuals. In this case, we would predict that subordinates have higher GCs levels than dominants during breeding periods and then take longer to return to non-breeding baseline. Whether either are the case has not yet been explored, but would be predicted by the stress-induced reproductive skew hypothesis (Creel, 2001; Young et al., 2006).

The broad aim of this study was to use fecal samples collected from free-ranging cooperative female meerkats across an 11-year period, to quantify within- and among-female variation in fecal glucocorticoid metabolites (fGC) during breeding and to assess the links among reproductive rate, dominance status and fGC (see below). Meerkats are small (<1 kg) social carnivores that reproduce cooperatively in groups of 2 to 40 individuals (mean ± sd = 16.7 ± 8.6) in arid zones of southern Africa. Groups generally consist of a dominant pair and overlapping generations of relatives that help to rear offspring (Clutton-Brock et al., 2001, 2002). Dominant females produce ~ 80% of offspring and subordinate breeding is biased toward older and larger subordinates (Clutton-Brock et al., 2001; Russell et al., 2004; Young et al., 2008). Gestation lasts ~70 days (Doolan and Macdonald, 1997), with increases in gonadal steroids from 35 days before birth (Moss et al., 2001; Fig. 1a). Some mothers can achieve a high reproductive rate by exhibiting a postpartum estrus (Moss et al., 2001); conceiving within days of parturition (Russell et al., 2003). We refer to these cases as 'overlapping breeding events' (Fig. 1b, scenario 1). Females breed up to four times a year (mode = 2 for dominants), deliver one to six pups per litter (mean ± sd = 3.73 ± 1.49) and lactate for about 35 days post-parturition (± 10 days, Russell et al., 2003). Meerkats therefore provide an ideal opportunity to test how adrenal activity during breeding varies between dominant and subordinate females, and how this is modified by reproductive rate (Fig. 1).

Specifically, we sought to document within- and among-female variation in fGC levels during discrete breeding events, characterized by a preconception and two pregnancy phases (Fig. 1), and to investigate individual characteristics that account for this variation. To this end, we first characterize variation in fGC within breeding events within females, among breeding events within females, among females and among social groups. Second, we describe fGC variation over the course of a breeding event and investigate whether patterns of variation associate with current reproductive rate. Third, we investigate the effect of dominance status and age on fGC levels throughout breeding events. Finally, we test whether individuals were consistent in their relative levels of fGC throughout breeding, such that fGC levels preconception predict levels during gestation and whether levels during early pregnancy predict those in late pregnancy. We controlled for several potential confounding factors, including sampling biases, female body mass and the socio-ecology of the group from which samples were collected.

Methods

We studied habituated meerkats from 1997 to 2008 at the Kuruman River Reserve in the South African Kalahari Desert (see Clutton-Brock et al., 1999; Russell et al., 2002 for details of habitat and climate). A total of 15 meerkat groups, numbering two to 40 marked individuals (mean = ~16), were followed every 1 to 5 days to monitor individual life-history and body mass, and to collect fecal samples, although groups were monitored daily when pregnant females were due to give birth. Body mass was determined before foraging each morning by enticing meerkats onto digital top-pan balances (± 1 g) using crumbs of hard-boiled egg (e.g. Clutton-Brock et al., 2002). Pregnancy was identified about 4 weeks after conception by swelling of the abdomen and nipples and by an increase in body mass. Parturition (± 1 day) was identified by a combination of sudden female mass loss and the presence of babysitters at the burrow (Clutton-Brock et al., 2001). Conception date was back-calculated to 70 days before birth (Doolan and Macdonald, 1997; Fig. 1). Based on the timing of increases in gonadal hormones following conception (Moss et al., 2001) and the timing of the main fetal growth phase during pregnancy (Russell et al., 2003), a breeding event was defined here by three 35-day phases (Fig. 1): (i) preconception (day −35 to −1); (ii) early pregnancy (day 1 to 35 of gestation); and (iii) late pregnancy (day 36 to 70). Those three phases allowed us to detail the increased adrenal activity during gestation and to account for the potential effects of consecutive overlapping breeding events on fGC levels (Fig. 1). Females reproducing at a high rate could conceive within 35 days of parturition and thereby would be both in late pregnancy and preconception (modal timing between parturition and postpartum conception is 9 days in dominant females; Young et al., 2006). The preconception phase of females known not to be pregnant with the previous litter provided a non-breeding fGC "baseline" level: we found no difference in fGC levels between females not pregnant during the 35-day preconception phase (n = 79 samples from 33 females) and those that were not pregnant at other times of the year (n = 300 samples from 77 females) (General Linear Mixed Model with normal error structure on log-transformed fGC levels controlling for female identity, collection time of day and storage duration: $F_{1,269} = 1.10$, $p = 0.29$, effect ± se = −0.13 ± 0.12). Because females experiencing postpartum conception are in early pregnancy and lactation phases at the same time, lactation was not considered as a separate breeding phase in this study but was accounted for by including the presence of unweaned pups at the burrow as a fixed effect in all analyses. All animal-handling protocols were approved by the University of Pretoria,

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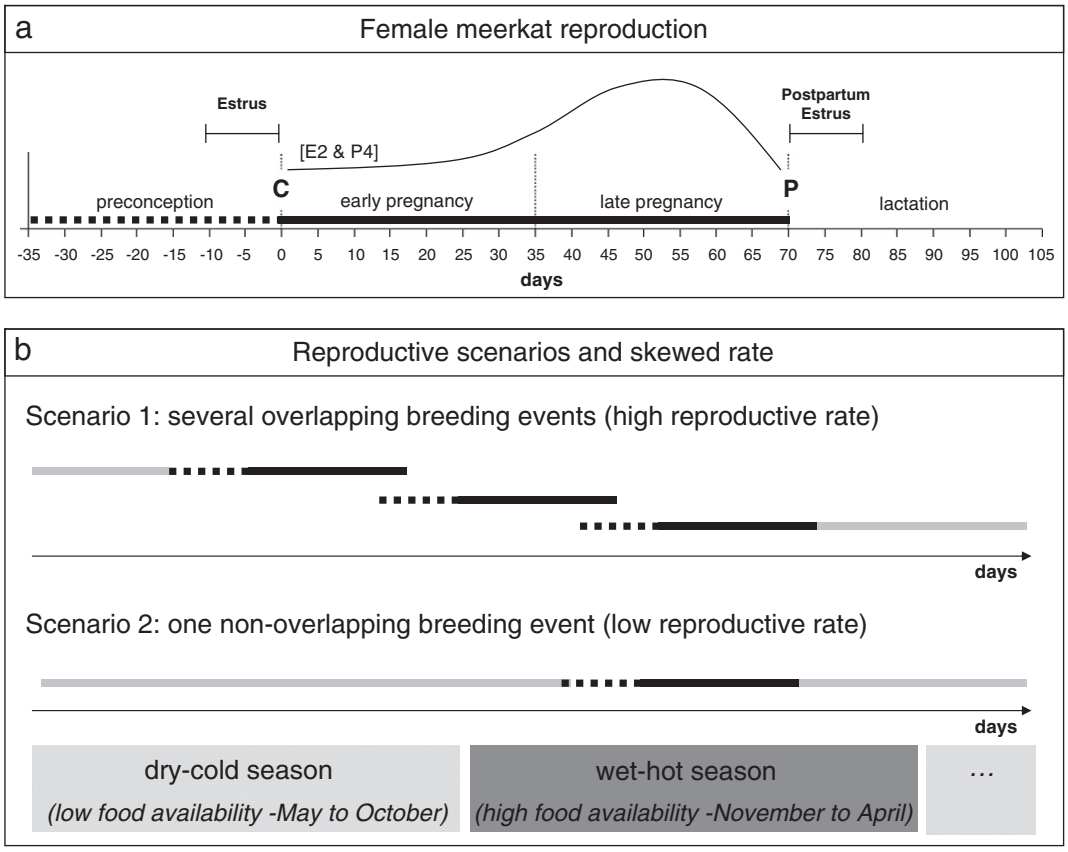

Fig. 1. The reproductive cycle of female meerkats. (a) Timing of estrus, conception (C), expected rise in estrogens and progestagens during pregnancy ([E2&P4] curve; Moss et al., 2001), parturition (P) and specific phases of each breeding event under study (preconception, dotted bold line, and early and late pregnancy, straight bold line). (b) Reproductive scenarios describing skewed reproductive rate among female meerkats which may or may not conceive immediately after parturition. Gray bold lines refer to non-breeding periods. In Scenario 1, a female overlaps in at least two consecutive breeding events. We distinguish climatic (yearly dry-cold and wet-hot seasons) from breeding seasons (yearly breeding season specific to each female and varying in duration and timing).

the University of Sherbrooke and the Smithsonian Institution ethic committees.

Sample collection and hormonal analyses

We studied fGC patterns only in females successfully breeding to term to preclude inadvertently including samples from females in the early stages of failed pregnancies. We thus assayed only those fecal samples collected within at least one of the three breeding phases of a given breeding event: preconception (n = 191 samples, 102 events, 44 females); early pregnancy (n = 188 samples, 113 events, 39 females); and late pregnancy (n = 200 samples, 96 events, 36 females). Overall, we assayed 579 fecal samples collected preceding or during 161 breeding events carried to term by 34 dominants (n = 473 samples, 132 events; mean ± sd samples per event = 3.5 ± 2.9) and 22 subordinates (n = 106 samples, 29 events; mean ± sd samples per event = 3.7 ± 5.3).

Fecal GC metabolites provide a pooled measure of adrenal steroids excreted over the past 24 to 48 h (Young et al., 2006), without the confounding stress of capture. Samples were collected whenever animals defecated, immediately placed on ice in thermos flasks and frozen at −20 °C within 5 h (mean ca. 2 h). Samples were shipped frozen to the Smithsonian Conservation Biology Institute in Front Royal, VA, USA. Steroid metabolites were extracted from samples using validated methodologies (Monfort et al., 1997). Briefly, fecal samples were freeze-dried (VirTis XL-70, SP Industries, New York), pulverized and thoroughly mixed. Fecal powder (0.18–0.19 g) was then combined with 6 ml of 100% ethanol, vortexed (10 s) and boiled (20 min) to extract steroid metabolites. After centrifugation (2500 *g*, 20 min), the supernatant was decanted into a tube and fully dried under a stream of compressed air; during evaporation, the vessel walls were rinsed twice with ethanol (4 then 2 ml). The residue was then redissolved in methanol (1 ml) and placed in an ultrasonic glass cleaner (5 min). A portion of the extractant was then diluted 1:50 in diluent buffer (pH 7.0) and frozen (−20 °C) for subsequent radioimmunoassay (RIA).

Concentrations of fGC were determined using a double-antibody ^{125}I RIA for corticosterone (ICN Biomedicals Inc., Costa Mesa, California, USA), which had been previously shown to most reliably measure adrenal activity (Young et al., 2006). Immunoreactive metabolites detected by the corticosterone antibody were defined generically as fecal glucocorticoid metabolites (fGC). The antiserum cross-reacts 100% with corticosterone, 0.34% with desoxycorticosterone, 0.10% with testosterone, 0.05% with cortisol, 0.03% with aldosterone, 0.02% with progesterone and <0.01% with all other steroids tested. Assays were conducted according to the instructions provided with the kit except that all reagent volumes were halved. Fecal extracts (1:50 dilution) were assayed (50 μl) in duplicate. Assay sensitivity was 25 ng/ml. Intra-assay coefficients of variation were <10% and inter-assay coefficients of variation for low- and high-dose internal controls were 8.8% and 6.7% for 20 assays performed from December 2005 to September 2008.

Statistical analyses

Analyses were conducted over an entire breeding event (preconception to parturition) and separately for each of the three specified breeding phases (Fig. 1a). These separate analyses have three main advantages. First, they facilitate interpretation of non-linear patterns in fGC levels over the entire breeding event. Second, they clarify the relative importance of individual characters for explaining variation

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in fGC at different times of a breeding event. Third, they permit investigations of the relationships between fGC levels during successive breeding phases.

In all analyses, fGC data were normalized using a natural logarithm transformation and fitted to a normal error structure in a General Linear Mixed Model (GLMM). In order to describe the hierarchical distribution of variation in the samples collected and interpret the data accordingly, we fitted four random terms to the models: (i) samples within a breeding event and within a female (hereafter referred to as inter-sample variation, n = (mean ± sd) 3.5 ± 3.2 samples per event); (ii) among events within females (hereafter inter-event variation, n = 2.8 ± 2.8 events per female); (iii) among events among females (hereafter inter-female variation, n = 4.9 ± 3.2 females per group); and (iv) among groups (hereafter inter-group variation). Random terms followed a nested structure, with samples being the lowest hierarchical level, and with higher levels being retained in final models only if they encompassed significant fGC variation after assessing the importance of fixed effects.

The fixed terms of interest outlined below were considered after controlling for the random terms retained as well as a number of potentially confounding fixed effects. Fixed effects with a potentially confounding influence included: storage duration, collection time-of-day, body mass and socio-ecological factors prevailing at the time of breeding and of sample collection. Because steroid metabolites can degrade over time (Schwartz and Monfort, 2008), we fitted as a fixed co-variate the number of days between sample collection and assay. To control for circadian variation in fGC, sample collection time was coded as morning (am: 06:00–12:00) and evening (pm: 15:00–20:00) and fitted as a two-level fixed co-factor in all analyses. Because female meerkats are more likely to breed and to become dominant when heavy (Clutton-Brock et al., 2006), we considered the potential association between body mass and fGC levels, although interpretation of mass results is difficult in a pregnant mammal. The potentially confounding socio-ecological factors prevailing at the time of sampling included: climatic season (wet-hot vs. dry-cold); group size (i.e. individuals ≥6 months old); number of breeding-aged females in the group (≥8 months); and pup presence at the burrow. We also considered the occurrence of concurrent female gestations in the group and the number of females evicted during the entire breeding event or phase in the case of phase-specific analyses. Only those confounding terms that accounted for significant variation in a given model were retained and their retention is indicated through the presentation of their statistical significance in the Results.

All analyses contained four fixed terms of primary interest where relevant. Although fixed terms of interest were also dropped from the final model when not significant, we report their effect when added to the final model individually in all cases. The primary fixed effects of interest in all models included: (1) timing into a discrete breeding event; (2) whether or not the event overlapped with other events; (3) female age; (4) dominance status. Timing of sample collection within a breeding event characterized maternal adrenal activity with days into breeding fitted as linear, quadratic and cubic functions to account for possible non-linearity; days varied from −35 to 70 in the analysis of all breeding data, and from 1 to 35 for each of the phase-specific analyses. Whether or not a female was late pregnant during the preconception phase characterized individual reproductive rate and was fitted as a two-level factor in all analyses, except during the late pregnancy phase where we fitted instead whether or not she was also preconceptive. A high reproductive rate resulting from postpartum conception is defined by the reproductive overlap of two phases of reproduction; a low reproductive rate is defined by no reproductive overlap (Fig. 1). Individual age (±1 d), was determined by birth dates in the study population. Dominance status was coded as dominant or subordinate with only one female per group being dominant (Kutsukake and Clutton-Brock, 2006). To disentangle the effect of reproductive rate and dominance status on among female variation in fGC pattern over time, we tested for interactions between timing into breeding and reproductive overlap, timing into breeding and dominance status, and reproductive overlap and dominance status.

Finally, we tested whether fGC levels preconception predict levels during gestation and whether levels during early pregnancy predict those in late pregnancy. A second analysis of fGC levels during early pregnancy was thus performed on a restricted data set where we could fit mean levels during the preconception phase of the same litter. Likewise, a second analysis of fGC levels during late pregnancy was performed on a restricted data set where we could fit mean levels during the preconception and early pregnancy phases of the same litter.

Statistical analyses were conducted with R (R Development Core Team, 2008). Normality was determined using quantile plots, frequency histograms and Shapiro–Wilk normality tests. The explanatory power of models and the individual fixed terms were considered in terms of the proportion of variance explained and was calculated by measuring the difference in residual variance including only random terms versus both random and fixed explanatory terms. The percentage of variation presented reflects the proportion of random variance explained by fixed effects not the proportion of overall total variance explained. Fixed terms and their two-way interactions were retained when they influenced the explanatory power of the model. Effect sizes were calculated by comparing the explanatory power of GLMMs including and excluding a term of interest in an ANOVA. Type III sum of square error structures (i.e. marginal error structure in R) are presented to control for co-variation among fixed terms (Pinheiro and Bates, 2000). Orthogonal polynomials were tested to control for collinearity among polynomial degrees (Shacham and Brauner, 1997). All statistical tests were two-tailed. Means and standard errors when presented in the text are predicted by GLMMs, and back-transformed to provide values in fGC ng/g of dry feces.

Results

Variation in fGC and variance explained

The variation in fGC levels recorded within the same female during a given breeding event was substantially greater than that recorded within females across different breeding events, among females, or among groups. Overall, ~87% of the variation in fGC levels occurred among samples from within the same female within a breeding event, ~9% occurred among breeding events of the same female, and ~4% occurred among individual females (see Table 1 for proportion of fGC variation explained at each hierarchical level; Fig. 2). The distribution of variation in fGC levels observed within each specific breeding phase closely mirrored the patterns outlined above in the analysis of all breeding data, with a notable exception (Table 1): during preconception phases, a larger share of the variation, i.e. ~32% vs. ~9%, was found among breeding events of the same female.

Effect of gestation and reproductive rate

The predicted mean levels of fGC halved during preconception, from ~157 to 87 ng/g of feces, then tripled by parturition, reaching ~305 ng/g of feces (Table 2; see Fig. 2a for raw data). These predicted mean values were obtained after controlling for positive effects of sample storage time ($F_{1,408} = 14.25$, $p < 0.001$) and body mass ($F_{1,408} = 32.94$, $p < 0.001$), negative effects of the number of eviction events ($F_{1,108} = 4.34$, $p = 0.04$) and differences associated with collection time of day (*am*<*pm*: $F_{1,408} = 21.48$, $p < 0.001$), climatic season (*wet/hot*<*dry/cold*: $F_{1,408} = 12.19$, $p < 0.001$) and occurrence of concurrent gestations (*no*<*yes*: $F_{1,108} = 3.82$, $p = 0.05$). The above average fGC pattern however masked a more complex relationship between timing into a breeding event and fGC levels that was influenced by whether or not females re-conceived within 35 days following parturition (i.e. by reproductive overlap) (Table 2a).

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Table 1
Proportion of variance in fGC excretion within breeding events (inter-samples), among events of a same female (inter-breeding events), and among females across all breeding events (inter-females). Inter-group variance was null in all cases and was thus not presented. Results are presented for analysis of all data (i.e., across a 105-day breeding event) and the phase-specific analyses. These data represent the proportion of variance explained only by random terms (i.e., by hierarchical levels) and the proportion of this variance at each hierarchical level that is explained by fixed effects (in parentheses).

Models	% variance in fecal glucocorticoid metabolites		
	Inter-samples	Inter-events	Inter-females
All breeding data	86.9 (37.1)	8.78 (51.0)	4.3 (0.0)
Preconception	68.2 (21.9)	31.79 (57.8)	0.0 (–)
Early pregnancy	90.5 (18.1)	4.97 (0.0)	4.5 (99.9)
Late pregnancy	89.3 (14.8)	3.86 (99.9)	6.9 (59.0)

The interaction between reproductive overlap and timing into breeding was clarified by investigating fGC variation within different phases of a breeding event. Females that were pregnant during the 35-day preconception phase of the forthcoming litter, had twice the fGC levels of those that were not pregnant within 35 days of conception (Table 2b). In all females, fGC levels then decreased near conception (Table 2b), although those pregnant preconception continued their decrease in fGC during early pregnancy (Table 2c). Fecal GC levels generally increased during late pregnancy, although females that conceived again postpartum excreted 3.5 times less fGC at parturition compared to females that did not conceive postpartum (Table 2d). In summary, females that were not pregnant during either the preconception or postpartum intervals (low reproductive rate with no reproductive overlap; Fig. 1b) excreted low fGC levels during preconception and early pregnancy, and then showed an increase in fGC that peaked at parturition (Fig. 3). In contrast, fGC in females that were pregnant preconception and postpartum (high reproductive rate with three consecutive breeding events; Fig. 1b) were initially high, declined throughout preconception and early pregnancy, then increased through late pregnancy before declining within 15 days of parturition (Fig. 3).

No effect of age or dominance status

Dominant and subordinate females did not differ in their fGC breeding profiles. We found no association of either age or dominance status with variation in fGC levels before and during breeding, after controlling for all significant effects outlined above (Table 2; Fig. 4). In addition, we found no interactions between dominance status and reproductive rate

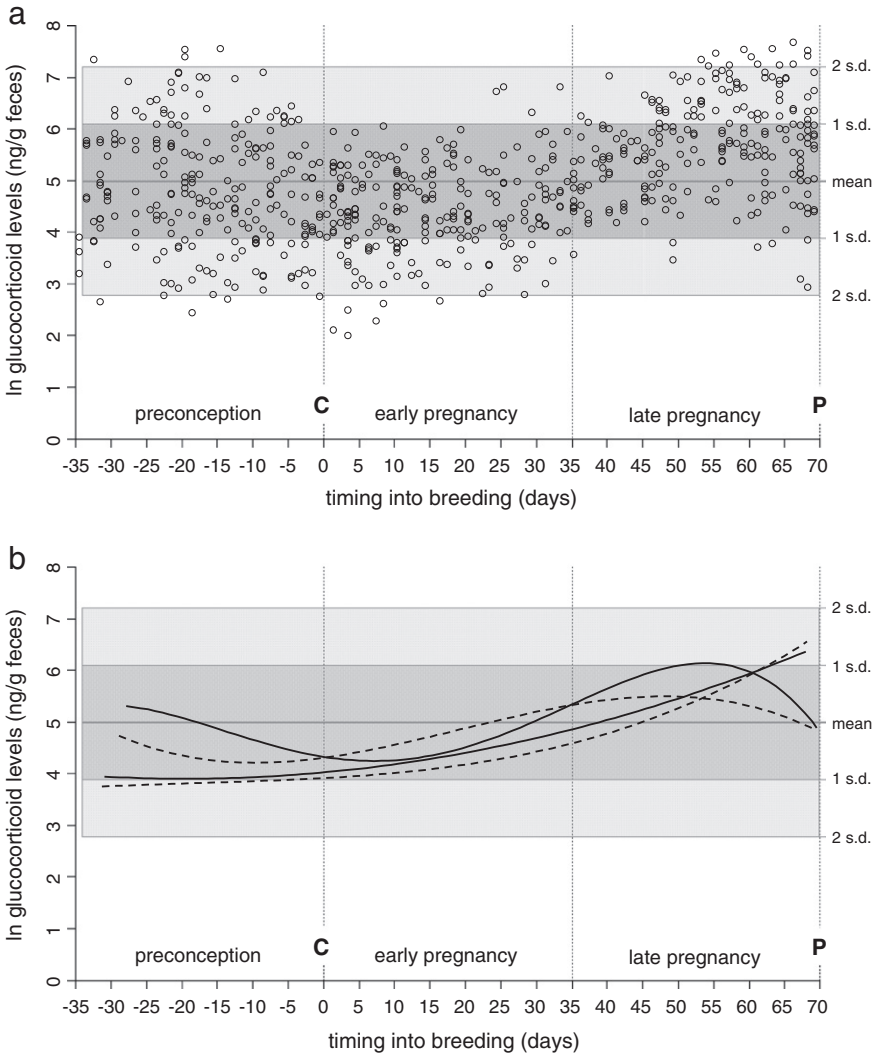

Fig. 2. Variation in fGC levels during a discrete breeding event, i.e. from day −35 to +70. (a) Log transformed raw data with mean ±1 and 2 standard deviations calculated for all samples included in this study. (b) Example of fGC variation among female meerkats and among breeding events with fGC profiles represented by respective best fit regression lines for a subset of two females over two separate breeding events each (females selected with most complete fGC profile); one female is illustrated by the continuous line, the other by the dashed line; mean and ±1 and 2 s.d. are calculated for all samples included in this study. Conception and parturition are identified with the letters C and P.

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Table 2
Factors affecting fGC of female meerkats during: (a) an entire breeding event (day −35 to 70); (b) preconception (day −35 to -1); (c) early pregnancy (day 1 to 35); and (d) late pregnancy (day 36 to 70). Only terms of interest and their interactions are presented; sampling, body mass and socio-ecological confounding effects are presented in the Results section.

GLMMs	*df*	Effect ± SE	F	p-Value
(a) All breeding data				
Constant[a]	1,408	4.03 ± 0.53	57.00	<0.001
Timing into breeding (B)				
Days B	1,408	−0.063 ± 0.011	30.16	<0.001
Days B^2	1,408	6.5E-4 ± 1.0E-4	38.19	<0.001
Reproductive overlap				
Pregnant preconception (*no*<*yes*)	1,108	−1.40 ± 0.31	20.61	<0.001
Pregnant preconception*Days B	1,408	0.039 ± 0.012	10.51	0.001
Pregnant preconception*Days B^2	1,408	−3.2E-4 ± 1.1E-4	9.07	0.003
Age (days)	1,407	−4E-5 ± 6.7E-5	0.36	0.55
Dominance status (subordinate, dominant)	1,407	See Fig. 4	0.005	0.94
Dominance*Pregnant preconception	1,407	0.14 ± 0.26	0.28	0.58
Dominance*Days B	1,407	1.1E-4 ± 3.8E-3	8.1E-4	0.98
(b) Preconception				
Constant[a]	1,116	5.37 ± 0.20	689.23	<0.001
Timing into preconception (Days PC)	1,116	−0.025 ± 0.007	13.52	<0.001
Reproductive overlap		See Fig. 3		
Pregnant preconception (*no*<*yes*)	1,57	−0.62 ± 0.19	10.82	0.002
Age (days)	1,115	−7.2E-5 ± 1.1E-4	0.47	0.49
Dominance status (subordinate, dominant)	1,115	See Fig. 4	2.65	0.10
Dominance*Pregnant preconception	1,56	0.53 ± 0.45	1.38	0.24
Dominance*Days PC	1,115	0.013 ± 0.016	0.67	0.41
(c) Early pregnancy				
Constant[a]	1,104	4.41 ± 0.13	1113.77	<0.001
Timing into early pregnancy (Days EP)	1,104	−0.023 ± 0.006	14.67	0.002
Reproductive overlap		See Fig. 3		
Pregnant preconception (*no*<*yes*)	1,73	−0.23 ± 0.23	1.05	0.31
Pregnant preconception*Days EP	1,104	0.029 ± 0.011	6.87	0.010
Age (days)	1,103	−2.5E-5 ± 6.6E-5	0.15	0.70
Dominance status (subordinate, dominant)	1,72	See Fig. 4	0.43	0.51
Dominance*Pregnant preconception	1,72	−0.29 ± 0.28	1.12	0.29
Dominance*Days EP	1,103	−0.012 ± 0.012	0.89	0.35
(d) Late pregnancy				
Constant[a]	1,99	5.23 ± 0.33	252.54	<0.001
Timing into late pregnancy (LP)				
Days LP	1,99	0.031 ± 0.037	0.73	0.39
Days LP^2	1,99	2.3E-4 ± 9.8E-4	0.057	0.81
Reproductive overlap		See Fig. 3		
Conceptive postpartum (*no*>*yes*)	1,58	−1.20 ± 0.43	7.62	0.008
Conceptive postpartum*Days LP	1,99	0.15 ± 0.05	7.87	0.006
Conceptive postpartum*Days LP^2	1,99	−4E-3 ± 1.4E-3	8.60	0.004
Age (days)	1,98	−1.4E-4 ± 7.9E-5	2.97	0.09
Dominance status (subordinate, dominant)	1,57	See Fig. 4	1.50	0.23
Dominance*Conceptive postpartum	1,57	0.48 ± 0.53	0.82	0.37
Dominance*Days LP	1,98	0.013 ± 0.016	0.67	0.41

[a] Values presented for the constants are specific to each periods and are calculated in reference to samples collected in the morning (*am*), during the dry-cold season, when no pups are present at the burrow, when no concurrent gestation occurs and for females pregnant preconception (*yes*) and not conceptive postpartum. Data were collected from 1997 to 2008 at the Kuruman River Reserve, South Africa.

or between dominance status and timing into breeding (Table 2). Although dominant females are larger and heavier than subordinates in meerkats (Russell et al., 2004), dominance status failed to reach significance even following the exclusion of significant mass effect (i.e. dominance effect when excluding body mass in GLMMs presented in Table 2; all breeding data: $F_{1,407} = 1.35$, $p = 0.25$, effect ± se = −0.18 ± 0.15; preconception: $F_{1,115} = 0.36$, $p = 0.55$, effect ± se = −0.13 ± 0.22; early pregnancy: no significant mass effect; late pregnancy: $F_{1,57} = 0.47$, $p = 0.49$, effect ± se = 0.17 ± 0.26).

Consistency in fGC levels during breeding

Levels of fGC during preconception were positively correlated with levels during early pregnancy, but not during late pregnancy. Average fGC levels preconception accounted for 8% of variation in fGC levels during early pregnancy, after controlling for all other significant terms (restricted GLMM with preconception and early pregnancy data available for a same breeding event: $F_{1,23} = 9.25$, $p = 0.006$, effect ± se = 0.27 ± 0.09). Specifically, although fGC levels during the 35 days preceding conception did not predict significant fGC variation within early pregnancy phases, preconception levels explained 50% of among early pregnancy phases variation within females (i.e. 4% of overall fGC variation) and 100% of the among females variation (i.e. 4% of overall fGC variation).

Discussion

Longitudinal hormone monitoring of free-ranging female meerkats clarified the interplay among individual variation in fGC excretion during breeding, reproductive rate and dominance status. Levels of fGC were highly variable, encompassing two orders of magnitude, and most of this variation occurred within breeding events from preconception through to parturition. Levels of fGC were generally low at conception and increased markedly in the latter half of gestation to parturition, although females with postpartum conception showed significant reductions in fGC levels in the last two weeks of gestation. Our results

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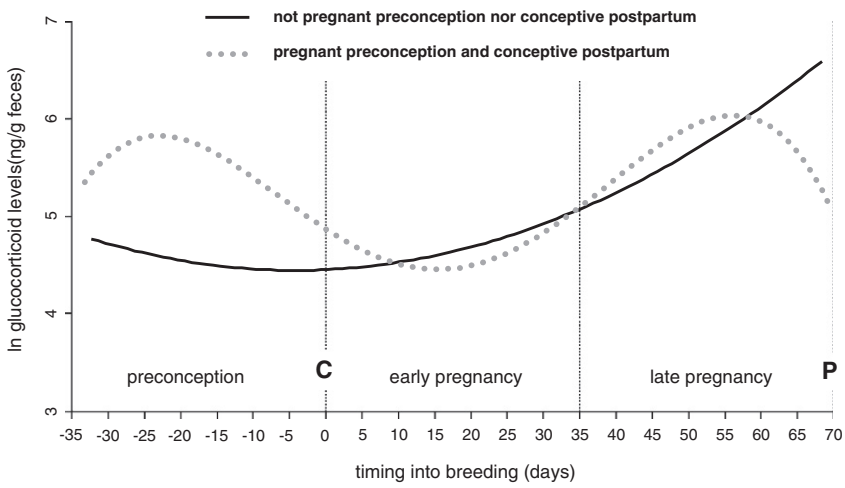

Fig. 3. Association between fGC levels and reproductive rates shown over a discrete breeding event as GLMM regression lines for adult female meerkats split into two breeder types: not pregnant preconception and not conceptive postpartum ($n = 153$ samples, 44 events and 20 mothers; $F_{2,40} = 12.95$, $p < 0.0001$); and pregnant preconception and conceptive postpartum ($n = 67$ samples, 24 events and 23 mothers; $F_{4,101} = 14.63$, $p < 0.0001$). Days of conception and parturition are respectively identified with the letter C and P.

conform to evidence that generally low GCs levels are required for successful conception and implantation (Douglas, 2010), but suggest that levels during late pregnancy can be very high without litters aborting. Finally, we found no evidence to suggest that dominants and subordinates differ in breeding GCs levels when experiencing the same reproductive rate. Nevertheless, because breeding is associated with higher fGC levels than non-breeding and because dominants breed at a higher rate that subordinates, dominants might often be observed to have higher adrenal activity than subordinates in cooperative breeders (Creel, 2001). We thus propose that studies investigating the role of adrenal activity in mediating reproductive skew would benefit from long-term sampling within and among individual females throughout non-breeding and breeding periods.

While longitudinal sampling of adrenal activity during periods of breeding is important for the reasons outlined above, care is required when interpreting the results. First, during gestation, GCs can arise from both maternal and feto-placental sources, with the relative contribution by the latter increasing as pregnancy proceeds (deM Fencl et al., 1980; Keller-Wood and Wood, 2001; Mastorakos and Ilias, 2000, 2003; Waddell, 1993). However, it is not unusual for the feto-placental unit to have little impact on maternal GCs levels, as is the case in rodents (Brunton et al., 2008). If feto-placental sources of GCs contribute significantly to maternal fGC levels in meerkats, we would expect fGC to peak at parturition. That fGC levels declined markedly in the last 2 weeks of gestation in female meerkats with postpartum conception suggests that any feto-placental sources of GCs in maternal feces have limited qualitative impact on maternal fGC levels. Second, relationships between maternal fGC and adrenal activity during lactation might be clouded by the stimulation of maternal adrenal activity by suckling offspring (Casey and Plaut, 2007) and the diffusion of maternal GCs into milk (Brummelte et al., 2010). That fGC levels declined during lactation in mothers with postpartum conception but showed non-significant tendencies to increase in those that were neither pre-conceptive nor early pregnant, suggests that neonatal stimulation/consumption of maternal GCs alone cannot account for maternal fGC levels during lactation (GLMM analysis on fGC levels during lactation in females that were neither pre-conceptive nor early pregnant, controlling for female identity, collection time of day and storage duration, $n = 93$ breeding events by 21 mothers: $F_{1,63} = 1.28$, $p = 0.21$; effect $\pm$ se $= 1.64 \pm 0.21$). Thus, our results suggest that the production of GCs from the feto-placental unit or the passive diffusion of maternal GCs into suckling offspring are insufficient to alter our observed patterns of maternal fGC qualitatively. This conclusion is further supported by findings that: (a) dominant females, which deliver and particularly suckle larger litters than subordinates (Russell et al., 2003), showed no differences with subordinates in fGC levels; and (b) that female mass was positively associated with fGC levels during preconception, suggests that similar correlations during late pregnancy cannot simply be attributed to larger/heavier litters.

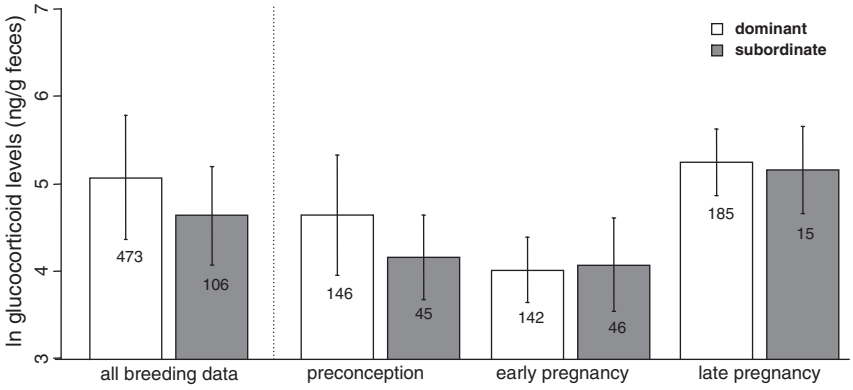

Fig. 4. Levels of fGC in subordinate and dominant meerkat females were not significantly different during a discrete breeding event and similarly during preconception and pregnancy phases. Predicted means and standard deviations are presented with sample sizes, after controlling for significant terms of interest and confounding sampling, body mass and socio-ecological factors.

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That postpartum conception was associated with substantial reductions in fGC during late pregnancy is intriguing because maternal GCs are also expected to rise to parturition to facilitate late fetal development (Atkinson and Waddell, 1995; Mastorakos and Ilias, 2003). This result cannot be explained by a relationship between postpartum conception and small litter sizes, because we have failed to find a relationship between litter size (delivered or weaned) and birth-intervals, and dominants, which show lower post-birth pup mortality than subordinates, have shorter, not longer, birth intervals (Russell et al., 2003). Indeed, we found little general evidence of a simple relationship between maternal energy requirements and fGC (Krasnow and Steiner, 2006). Maternal fGC levels showed earlier peaks in gestation and declined both late in pregnancy and during lactation in mothers experiencing postpartum conception, when energetic requirements would be at their greatest. The mechanisms underpinning these contrasting patterns in maternal fGC during postpartum and non-postpartum conceptions have yet to be elucidated. One possibility is that reduction in fGC arises from the antagonist effect of increasing estrogens associated with postpartum conception which are known to interfere with the hypothalamic–pituitary–adrenal (HPA) axis (Douglas, 2010; Mastorakos and Ilias, 2003). Whether such potential effects of estrogens arise from a physiological or behavioral strategy on the part of mothers to reduce inter-birth intervals (Breuner, 2008; Meylan and Clobert, 2005; Nguyen et al., 2008), or inter-birth intervals are reduced as a consequence of patterns of fGC during late pregnancy, is currently unclear (Wagenmaker et al., 2009).

Previous research investigating the role of GCs in mediating reproductive suppression has been conducted outside the breeding season or involved non-breeders (Creel, 2001, 2005). The initial conclusion from this research is that in cooperative breeders, dominant females do not attempt to suppress the reproductive functions of subordinates through inducing chronic elevation in GCs (Creel, 2001). However, Young et al. (2006) noted that comparisons of adrenal activity among dominant and subordinate females during periods of non-breeding might be problematic, if pregnant dominants induce chronic stress in subordinates, for example by evicting them from the group. In support, Young et al. (2006) found that subordinates had double the levels of fGC when evicted (~200 ng/g feces), and during this time either failed to conceive or aborted if pregnant. In our study, the majority of conceptions occurred with fGC levels of under 100 ng/g feces, even among those with a postpartum conception, although conception and implantation did appear to be possible with levels as high as ~400 ng/g feces in some instances. These results conform to the general trend that successful conception and implantation require low adrenal activity (Douglas, 2010; Nakamura et al., 2008), and are broadly supportive of the possibility that sustained levels of ~200 ng/g feces might preclude conception in most females (Young et al., 2006). However, whether or not high adrenal activity is the mediating factor behind abortions as suggested by Young et al. (2006) is less clear, although GCs are proposed to cause abortion in other mammal species (Arck, 2001; Douglas, 2010; Magiakou et al., 1997; Nakamura et al., 2008; Nepomnaschy et al., 2006). In meerkats, identification of pregnancy is difficult until the second half of gestation (i.e. late pregnancy in this study). Even during the first week of late pregnancy, fGC levels already averaged ~200 ng/g feces and levels of up to ~400 ng/g feces were common; with this rising to ~1000 ng/g feces in some females by the start of the final third of gestation. That all our females carried to term despite high levels of fGC, in conjunction with known reduced sensitivity of maternal HPA axis to stressors toward parturition in other species (Brunton et al., 2008; Douglas et al., 2003; Johnstone et al., 2000), suggests that further work is required to test the link between adrenal activity and abortions.

We found no evidence to suggest that dominants and subordinates differ in their levels or patterns of fGC, either during preconception, early pregnancy or late pregnancy. Young et al. (2008) failed to find a difference between fGC levels in dominant versus subordinate meerkats during non-breeding periods. That we found no difference between dominants and subordinates during preconception, a period when fGC levels are similar to non-breeding periods, supports this earlier finding of Young et al. (2008). In addition, our results that dominants and subordinates reproducing at a similar rate had similar mean fGC levels during preconception and pregnancy phases, and similar slopes with advancement of each phase, suggest that, at least among those that carried successfully to term, there is no indication that dominants and subordinates significantly differ in their adrenal activity. Finally, we found no evidence to suggest that dominants and subordinates differ in their fGC profiles in association with reproductive rate, suggesting that the low reproductive rate commonly associated with subordinates does not arise due to higher "stress" involved with reproduction. This conclusion has been reached previously, because dominants and sexually mature subordinates do not differ in their foraging success (Russell et al., 2004). Taken together, these results suggest that at least among those females considered in this study, subordinates are not constrained from reproduction due to inferior condition or elevated physiological costs; implying that either dominant suppression or self-restraint, when the probability of breeding successfully is low, mediates the incidence of subordinate reproduction in meerkats.

In conclusion, our study has a number of important implications. First, we suggest that studies of cooperative breeders showing dominants to have higher GCs levels than subordinates might partly reveal an artifact of high reproductive rates and either reflect increased levels directly involved with aspects of reproduction or the consequences of reproduction in the form of compensatory foraging effort (Russell et al., 2004). Second, increases in adrenal activity during gestation are suggested to be required for successful fetal development (Atkinson and Waddell, 1995; Mastorakos and Ilias, 2003). That female meerkats showed substantial reductions late in pregnancy when followed by a postpartum conception, either calls the generality of this effect into question or suggests that litters followed by postpartum conception should have impaired growth and/or development. Concomitantly, however, females with a postpartum conception conceived with higher fGC levels and higher levels during preconception were associated with higher levels during early pregnancy. The consequences of high reproductive rates for mothers and offspring, and whether mothers might use varying rates to vary offspring phenotype are currently unclear (Russell and Lummaa, 2009).

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References

Abbott, D.H., Saltzman, W., Schultz-Darken, N.J., Smith, T.E., 1997. Specific neuroendocrine mechanisms not involving generalized stress mediate social regulation of female reproduction in cooperatively breeding marmoset monkeys. Ann. N. Y. Acad. Sci. 807, 219–238.

Arck, P.C., 2001. Stress and pregnancy loss: role of immune mediators, hormones and neurotransmitters. Am. J. Reprod. Immunol. 46, 117–123.

Atkinson, H.C., Waddell, B.J., 1995. The hypothalamic–pituitary–adrenal axis in rat pregnancy and lactation: circadian variation and interrelationship of plasma adrenocorticotropin and corticosterone. Endocrinology 136, 512–520.

Beekman, M., Ratnieks, F.L.W., 2003. Power over reproduction in social hymenoptera. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 358, 1741–1753.

Bennett, N.C., 1994. Reproductive suppression in social *Cryptomys damarensis* colonies — a lifetime of socially-induced sterility in males and females (*Rodentia*, *Bathyergidae*). J. Zool. 234, 25–39.

Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009. Do baseline glucocorticoids predict fitness? Trends Ecol. Evol. 24, 634–642.

Breuner, C., 2008. Maternal stress, glucocorticoids, and the maternal/fetal match hypothesis. Horm. Behav. 54, 485–487.

Brummelte, S., Schmidt, K.L., Taves, M.D., Soma, K.K., Galea, L.A.M., 2010. Elevated corticosterone levels in stomach milk, serum, and brain of male and female offspring after maternal corticosterone treatment in the rat. Dev. Neurobiol. 70, 714–725.

Brunton, P.J., Russell, J.A., Douglas, A.J., 2008. Adaptive responses of the maternal hypothalamic–pituitary–adrenal axis during pregnancy and lactation. J. Neuroendocrinol. 20, 764–776.

Cant, M.A., 2011. The role of threats in animal cooperation. Proc. R. Soc. Lond. B. Biol. Sci. 278, 170–178.

Casey, T.M., Plaut, K., 2007. The role of glucocorticoids in secretory activation and milk secretion, a historical perspective. J. Mammary Gland Biol. Neoplasia 12, 293–304.

Choe, J.C., Crespi, B.J., 1997. The Evolution of Mating Systems in Insects and Arachnids. Cambridge University Press, Cambridge, UK.

Clutton-Brock, T.H., Gaynor, D., McIlrath, G.M., Maccoll, A.D.C., Kansky, R., Chadwick, P., Manser, M., Skinner, J.D., Brotherton, P.N.M., 1999. Predation, group size and mortality in a cooperative mongoose, *Suricata suricatta*. J. Anim. Ecol. 68, 672–683.

Clutton-Brock, T.H., Brotherton, P.N.M., Russell, A.F., O'Riain, M.J., Gaynor, D., Kansky, R., Griffin, A., Manser, M., Sharpe, L., McIlrath, G.M., Small, T., Moss, A., Monfort, S., 2001. Cooperation, control, and concession in meerkat groups. Science 291, 478–481.

Clutton-Brock, T.H., Russell, A.F., Sharpe, L.L., Young, A.J., Balmforth, Z., McIlrath, G.M., 2002. Evolution and development of sex differences in cooperative behavior in meerkats. Science 297, 253–256.

Clutton-Brock, T.H., Hodge, S.J., Spong, G., Russell, A.F., Jordan, N.R., Bennett, N.C., Sharpe, L.L., Manser, M.B., 2006. Intrasexual competition and sexual selection in cooperative mammals. Nature 444, 1065–1068.

Creel, S., 2001. Social dominance and stress hormones. Trends Ecol. Evol. 16, 491–497.

Creel, S., 2005. Dominance, aggression, and glucocorticoid levels in social carnivores. J. Mammal. 86, 255–264.

Dantzer, B., McAdam, A.G., Palme, R., Fletcher, Q.E., Boutin, S., Humphries, M.M., Boonstra, R., 2010. Fecal cortisol metabolite levels in free-ranging North American red squirrels: assay validation and the effects of reproductive condition. Gen. Comp. Endocrinol. 167, 279–286.

de Kloet, E.R., Oitzl, M.S., Joels, M., 1999. Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci. 22, 422–426.

deM Fencl, M., Stillman, R.J., Cohen, J., Tulchinsky, D., 1980. Direct evidence of sudden rise in fetal corticoids late in human gestation. Nature 287, 225–226.

Dhabhar, F.S., 2002. A hassle a day may keep the doctor away: stress and the augmentation of immune function. Integr. Comp. Biol. 42, 556–564.

Dingemanse, N.J., Edelaar, P., Kempenaers, B., 2010. Why is there variation in baseline glucocorticoid levels? Trends Ecol. Evol. 25, 261–262.

Doolan, S.P., Macdonald, D.W., 1997. Breeding and juvenile survival among slender-tailed meerkats (*Suricata suricatta*) in the south-western Kalahari: ecological and social influences. J. Zool. 242, 309–327.

Douglas, A.J., 2010. Baby on board: do responses to stress in the maternal brain mediate adverse pregnancy outcome? Front. Neuroendocrinol. 31, 359–376.

Douglas, A.J., Brunton, P.J., Bosch, O.J., Russell, J.A., Neumann, I.D., 2003. Neuroendocrine responses to stress in mice: hyporesponsiveness in pregnancy and parturition. Endocrinology 144, 5268–5276.

Faulkes, C.G., Abbott, D.H., 1997. The physiology of a reproductive dictatorship: regulation of male and female reproduction by a single breeding female in colonies of naked mole-rats. In: Solomon, N.G., French, J.A. (Eds.), Cooperative Breeding in Mammals. Cambridge University Press, Cambridge, pp. 302–334.

Hager, R., Jones, C.B., 2009. Reproductive skew in vertebrates. Proximate and Ultimate Causes. Cambridge University Press, Cambridge.

Johnstone, H.A., Wigger, A., Douglas, A.J., Neumann, I.D., Landgraf, R., Seckl, J.R., Russell, J.A., 2000. Attenuation of hypothalamic–pituitary–adrenal axis stress responses in late pregnancy: changes in feedforward and feedback mechanisms. J. Neuroendocrinol. 12, 811–822.

Keller-Wood, M., Wood, C.E., 2001. Pituitary–adrenal physiology during pregnancy. Endocrinologist 11, 159–170.

Koenig, W.D., Dickinson, J.L., 2004. Ecology and Evolution of Cooperative Breeding in Birds. Cambridge University Press, Cambridge.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935.

Krasnow, S.M., Steiner, R., 2006. Physiological mechanisms integrating metabolism and reproduction. In: Neill, J.D. (Ed.), Knobil and Neill's Physiology of Reproduction. Elsevier Academic Press, St. Louis, MO, pp. 2553–2625.

Kutsukake, N., Clutton-Brock, T.H., 2006. Aggression and submission reflect reproductive conflict between females in cooperatively breeding meerkats Suricata suricatta. Behav. Ecol. Sociobiol. 59, 541–548.

Magiakou, M.A., Mastorakos, G., Webster, E., Chrousos, G.P., 1997. The hypothalamic–pituitary–adrenal axis and the female reproductive system. Ann. N. Y. Acad. Sci. 816, 42–56.

Mastorakos, G., Ilias, I., 2000. Maternal hypothalamic–pituitary–adrenal axis in pregnancy and the postpartum period — postpartum-related disorders. Ann. N. Y. Acad. Sci. 900, 95–106.

Mastorakos, G., Ilias, I., 2003. Maternal and fetal hypothalamic–pituitary–adrenal axes during pregnancy and postpartum. Ann. N. Y. Acad. Sci. 997, 136–149.

Meylan, S., Clobert, J., 2005. Is corticosterone-mediated phenotype development adaptive? Maternal corticosterone treatment enhances survival in male lizards. Horm. Behav. 48, 44–52.

Monfort, S.L., Wasser, S.K., Mashburn, K.L., Burke, M., Brewer, B.A., Creel, S.R., 1997. Steroid metabolism and validation of noninvasive endocrine monitoring in the African wild dog (*Lycaon pictus*). Zoo Biol. 16, 533–548.

Moss, A.M., Clutton-Brock, T.H., Monfort, S.L., 2001. Longitudinal gonadal steroid excretion in free-living male and female meerkats (*Suricata suricatta*). Gen. Comp. Endocrinol. 122, 158–171.

Nakamura, K., Sheps, S., Arck, P.C., 2008. Stress and reproductive failure: past notions, present insights and future directions. J. Assist. Reprod. Genet. 25, 47–62.

Nepomnaschy, P.A., Welch, K.B., McConnell, D.S., Low, B.S., Strassmann, B.I., England, B.G., 2006. Cortisol levels and very early pregnancy loss in humans. Proc. Natl. Acad. Sci. U. S. A. 103, 3938–3942.

Nguyen, N., Gesquiere, L.R., Wango, E.O., Alberts, S.C., Altmann, J., 2008. Late pregnancy glucocorticoid levels predict responsiveness in wild baboon mothers (*Papio cynocephalus*). Anim. Behav. 75, 1747–1756.

Pinheiro, J.C., Bates, D.M., 2000. Mixed-effects Models in S and S-Plus. Springer, New York.

R Development Core Team, 2008. R: A language and environment for statistical computing, in: R.F.f.S. (Ed.), Computing. Vienna, Austria.

Romero, L.M., Reed, J.M., 2008. Repeatability of baseline corticosterone concentrations. Gen. Comp. Endocrinol. 156, 27–33.

Russell, A.F., Lummaa, V., 2009. Maternal effects in cooperative breeders: from hymenopterans to humans. Proc. R. Soc. Lond. B. Biol. Sci. 364, 1143–1167.

Russell, A.F., Clutton-Brock, T.H., Brotherton, P.N.M., Sharpe, L.L., McIlrath, G.M., Dalerum, F.D., Cameron, E.Z., Barnard, J.A., 2002. Factors affecting pup growth and survival in co-operatively breeding meerkats (*Suricata suricatta*). J. Anim. Ecol. 71, 700–709.

Russell, A.F., Brotherton, P.N.M., McIlrath, G.M., Sharpe, L.L., Clutton-Brock, T.H., 2003. Breeding success in cooperative meerkats: effects of helper number and maternal state. Behav. Ecol. 14, 486–492.

Russell, A.F., Carlson, A.A., McIlrath, G.M., Jordan, N.R., Clutton-Brock, T., 2004. Adaptive size modification by dominant female meerkats. Evolution 58, 1600–1607.

Saltzman, W., Ahmed, S., Fahimi, A., Wittwer, D.J., Wegner, F.H., 2006. Social suppression of female reproductive maturation and infanticidal behavior in cooperatively breeding Mongolian gerbils. Horm. Behav. 49, 527–537.

Sapolsky, R.M., 1994. Individual-differences and the stress-response. Semin. Neurosci. 6, 261–269.

Sapolsky, R.M., 1999. Hormonal correlates of personality and social contexts: from non-human to human primates. In: Panter-Brick, C., Worthman, C. (Eds.), Hormones, Health, and Behaviour: A Socio-Ecological and Lifespan Perspective. Cambridge University Press, Cambridge, UK, p. 18.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.

Schwartz, M., Monfort, S., 2008. Genetic and endocrine tools for carnivore surveys. In: Long, R., Mackay, P., Zielinski, W., Ray, J. (Eds.), Noninvasive Survey Methods for Carnivores. Island Press, Washington DC, pp. 238–262.

Selye, H., 1956. The Stress of Life. McGraw-Hill, New York.

Shacham, M., Brauner, N., 1997. Minimizing the effects of collinearity in polynomial regression. Ind. Eng. Chem. Res. 36, 4405–4412.

Solomon, N.G., French, J.A., 1997. Cooperative Breeding in Mammals. Cambridge University Press, Cambridge, UK.

Stacey, P.B., Koenig, W.D., 1990. Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior. Cambridge University Press, Cambridge, UK.

Trainer, P.J., 2002. Corticosteroids and pregnancy. Semin. Reprod. Med. 20, 375–380.

Waddell, B.J., 1993. The placenta as hypothalamus and pituitary: possible impact on maternal and fetal adrenal function. Reprod. Fertil. Dev. 5, 479–497.

Wagenmaker, E.R., Breen, K.M., Oakley, A.E., Pierce, B.N., Tilbrook, A.J., Turner, A.I., Karsch, F.J., 2009. Cortisol interferes with the estradiol-induced surge of luteinizing hormone in the ewe. Biol. Reprod. 80, 458–463.

Whittle, W.L., Patel, F.A., Alfaidy, N., Holloway, A.C., Fraser, M., Gyomorey, S., Lye, S.J., Gibb, W., Challis, J.R.G., 2001. Glucocorticoid regulation of human and ovine parturition: the relationship between fetal hypothalamic–pituitary–adrenal axis activation and intrauterine prostaglandin production. Biol. Reprod. 64, 1019–1032.

Williams, T.D., 2008. Individual variation in endocrine systems: moving beyond the 'tyranny of the Golden Mean'. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363, 1687–1698.

Wilson, E.O., 1971. The Insect Societies. Harvard University Press, Cambridge, MA.

Wingfield, J.C., Kitaysky, A.S., 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integr. Comp. Biol. 42, 600–609.

Young, A.J., Carlson, A.A., Monfort, S.L., Russell, A.F., Bennett, N.C., Clutton-Brock, T., 2006. Stress and the suppression of subordinate reproduction in cooperatively breeding meerkats. Proc. Natl. Acad. Sci. U. S. A. 103, 12005–12010.

Young, A.J., Monfort, S.L., Clutton-Brock, T.H., 2008. The causes of physiological suppression among female meerkats: a role for subordinate restraint due to the threat of infanticide? Horm. Behav. 53, 131–139.

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